Learning to breathe?

Feedforward regulation of the inspiratory motor drive

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Claims have been made that breathing is in part controlled by feedforward regulation. In a classical conditioning paradigm, we investigated anticipatory increases in the

inspiratory motor drive as measured by inspiratory occlusion pressure (P100). In an acquisition phase, an experimental group (N=13) received a low-intensity resistive load (5 $cmH_2O/l/s$) for three consecutive inspirations as Conditioned Stimulus (CS), preceding a load of a stronger intensity (20 $cmH_2O/l/s$) for three subsequent inspirations as Unconditioned Stimulus (US). The control group (N=11) received the low-intensity load for 6 consecutive inspirations. In a post-acquisition phase both groups received the low-intensity load for 6 consecutive inspirations.

Responses to the CS-load only differed between groups during the first acquisition trials and a strong increase in P100 during the US-loads was observed, which habituated across the experiment. Our results suggest that the disruption caused by adding low to moderate resistive loads to three consecutive inspirations results in a short-lasting anticipatory increase in inspiratory motor drive.

KEYWORDS

Respiratory learning, inspiratory drive, occlusion pressure, resistive loads, interoceptive conditioning

1. Introduction

Behavioral control of breathing refers to the modulation of the breathing pattern that is not primarily related to metabolic requirements. This includes, for example, voluntary changes in breathing patterns such as during speech, and involuntary changes caused by variations in vigilance, emotions and cognitive activity (Gallego et al., 2001). Somjen (1992) proposed that learning mechanisms starting at an early age contribute to behavioral control. In early infancy breathing regulation is mainly controlled by feedback mechanisms in response to environmental or internal changes perturbing breathing, but with repeated perturbations the respiratory system would learn to anticipate and start responding to perturbations before they actually occur. Because of this anticipatory nature, Somjen (1992) used the term "feedforward regulation" to contrast it with feedback-based regulation of metabolic systems (see also Dworkin, 1993).

Anticipatory changes in breathing behavior have been documented for chemical perturbations, but studies on mechanical perturbations are sparse. An instance of the former may be the anticipatory increase in breathing when an increase in metabolism (CO_2 production) as a result of exercise is expected, the so-called "exercise hyperpnoea" (Fink et al., 1995; Mitchell et al., 1990; Tobin et al., 1986; Wood et al., 2003). During exercise, ventilation increases in proportion to O_2 consumption and CO_2 production, thus allowing a stabilization of blood gases. However, Wood et al. (2003) documented a decrease in PCO_2 starting already at onset of exercise, showing that the immediate respiratory response to exercise is not triggered by chemoreceptor feedback. Apparently, because of the narrow regulation of blood gas levels (Shea, 1997), a feedforward

regulation mechanism triggers an adaptive ventilatory response by anticipation of a forthcoming increase in PCO_2. This view is further confirmed by studies on imagined exercise or activated emotions, in which an augmented ventilation is observed without any associated movement or increase metabolism (Gallego et al., 1996; Van Diest et al., 2001).

The development of anticipatory control of breathing can easily be understood from an associative learning framework, or, classical conditioning. During conditioning, a neutral stimulus (CS, conditioned stimulus) becomes associated with a motivationally relevant stimulus (US, unconditioned stimulus), which elicits an unconditional response (UR). As a result, the CS acquires predictive value for the occurrence of the US and starts to elicit a conditioned response that is similar to the unconditioned response. For example, perturbations in arterial blood gases can be viewed as unconditioned stimuli (US) and internal or external stimuli entailing a predictive value for such perturbations would function as conditioned stimuli (CS). Particularly interoceptive conditioning (IC) has been proposed as an important regulatory mechanism through which the body can anticipate and adapt to upcoming dysregulations (Dworkin and Dworkin, 1995). Interoceptive conditioning occurs when a sensation from within the body (CS) becomes a predictor of a significant disturbance (US). IC has been studied mainly in the context of drug tolerance and addiction (Sokolowska et al., 2002; Ramsay and Woods, 1997), of eating behavior and blood glucose regulation (Epstein et al., 2009; Dworkin, 1993; Woods and Ramsay, 2000) and in the context of blood pressure regulation (Dworkin and Dworkin, 1995; Razran, 1961, 2002). To date, no studies have investigated the potential

role that conditioning to interoceptive cues may play in the regulation of breathing behavior.

A few studies have investigated respiratory conditioning to *external* cues, however. For example, Nsegbe, Gallego and coworkers (Nsegbe et al., 1998) paired an odor (CS) with a hypoxic stimulus in rats. Their data show that the odor-CS, when presented alone in a subsequent test phase, elicited a conditioned increase in ventilation. In previous research, we have established a laboratory paradigm to study conditioning of human respiratory behavior in response to exteroceptive stimuli in humans. Predictable dysregulations of arterial pressure of CO_2 were established with a Pavlovian conditioning procedure in which inhalation of 7.5% or 20% CO_2 was used as the US (e.g., Fannes et al., 2008; Van den Bergh et al., 1995; Van den Bergh et al., 1997), reflexively triggering an increase in ventilation. When such increase in PCO_2 was preceded by an odor or a tone (CS), participants learned to increase their ventilation in response to the tone or the odor, that is, *in anticipation* to the metabolic dysregulation caused by inhaling CO_2. However, the observed effects were small and not consistent across studies, probably because of several methodological difficulties inherent to the use of CO_2-inhalation in a conditioning paradigm, such as the US qualities being dependent on the participants breathing behavior, the relatively slow rise of the aversive sensation and the long duration to wash out the increased PCO_2 levels after each trial.

In contrast with inhalation of CO_2-enriched air, mechanical disruptions as induced by adding resistive loads to the external breathing circuit may offer interesting opportunities to investigate learned breathing control in a more controlled way. Resistive loads have been used repeatedly as a respiratory challenge (Milic-Emili and Zin, 1986),

can be easily detected (Bloch-Salisbury and Harver, 1994; Davenport et al., 1986), and have a discrete on- and offset that can be tightly controlled in the laboratory. Resistive load breathing actually feels like one were to breathe through a straw. Adding them to an external breathing circuitry resembles a naturally occurring phenomenon, i.e., instances where our respiratory system must cope with obstructions and try to keep the airways open. The adaptive ventilatory response/reflex in response to inspiratory resistive loads is to augment the respiratory drive (UR) (Altose et al., 1976; Im Hof et al., 1986; Lopata et al. 1977; Poon, 1989). The dynamic response to loads is much faster than for CO_2-challenges: Altose et al. (1979) have reported an increase in respiratory drive from the second loaded breath onwards.

The central respiratory drive is the integrated output from the Central Nervous System (CNS) to the respiratory pump muscles, also called the summed motor output of the respiratory centers. A respiratory parameter generally used to measure the respiratory drive is P100 (Van Diest et al., 2008; Whitelaw and Derenne, 1993). P100 is the inspiratory occlusion pressure generated 100 msec after the onset of an inspiratory effort against a closed airway. P100 is the decrease in mouth pressure assumed to reflect the intra-thoracic negative pressure generated by the respiratory muscles. As mentioned previously, it reflects 'direct' cortical input to the respiratory controller. The more traditional respiratory parameters, like inspiratory volume or minute ventilation are influenced by mechanical factors involved in the transformation of respiratory motor neuron output into ventilation, such as airway resistance or elasticity of lungs and thorax (Whitelaw and Derenne, 1993). As the impedance of the system is changed, they no longer can be used to evaluate the output of the controller (Whitelaw and Derenne, 1993).

Mechanical perturbations such as breathing loads and occlusions were successfully introduced as CS or US in fear learning paradigms (Pappens et al., 2011; Pappens et al., 2012). In two experiments, Pappens and colleagues (2013) compared an interoceptive CS (non-aversive resistive load) with an exteroceptive CS (neutral picture) in a fear learning paradigm with an aversive, strong resistive load as the US. They found fear conditioned changes in volume-related breathing parameters. However, these effects were small and potentially confounded by the fear response itself, as fear typically augments ventilator output. Whether anticipatory changes in breathing pattern for mechanical perturbations can be established also in a non-fearful context remains unexplored. Interestingly, recent evidence in animals has shown that anticipatory alterations (inspiratory-related phrenic nerve activity) can be established by vagal stimulation or lung inflation in perfused brainstem preparations (Dutschmann et al., 2009), suggesting that the fear circuitry is not necessarily involved in anticipatory changes in the breathing pattern.

The present study aimed to explore whether interoceptive conditioning of inspiratory motor drive could be established throughout the contingent pairing of a small inspiratory load with a stronger inspiratory load. To this end, during a learning phase (acquisition) three breaths loaded with a low intensity load preceded three breaths with a stronger intensity load in the experimental group while in a control group six breaths loaded with a low intensity load were presented. In a post-learning (post-acquisition) phase both groups received six breaths loaded with the low intensity load. We expected that participants in the experimental group would learn to anticipatorily adapt to the strong load by increasing their inspiratory motor drive to the low-intensity load during

the learning phase and that this anticipatory response would wane during the post-learning phase.

2. Method

2.1. Participants

Thirty-three healthy participants (7 men and 26 women, mean age 18.7, range 18-21 years) volunteered to participate. Twenty-six participants were undergraduate students who participated in return for course credit. Seven other volunteers responded to local advertisements and were paid 10 €. A brief, custom-made health survey was administered to exclude participants when suffering from asthma or other respiratory diseases, cardiac diseases, epilepsy, anxiety disorders, and the use of medications that might suggest the presence of these conditions. No participants were positive on any of these. After exclusion due to technical problems (N= 6) or excessive variability in the P100 data (N= 3), the experimental and control group consisted of 13 and 11 participants, respectively. Each participant provided an informed consent. The experiment was approved by the ethical committees of the Faculty of Psychology and Educational Sciences and of the Faculty of Medical Sciences.

2.2. Materials

2.2.1. Subjective measures.

Prior to the conditioning procedure, participants completed the Dutch version of the Claustrophobia Scale (Rachman and Taylor, 1993; Van Diest et al., 2010) to measure fear of suffocation. This was done because previous work has suggested that interindividual

differences in Fear of Suffocation modulate the respiratory response to resistive loads (Pappens et al., 2012b; Alius et al., 2013).

Prior and following the conditioning procedure, participants rated each of both loads on the affective dimensions of valence, arousal and dominance with the Self-Assessment Manikin system (SAM, Bradley and Lang, 1994). Participants rated the loads on a 5-point scale, with the valence dimension ranging from pleasant (1) to unpleasant (5), the arousal dimension ranging from relaxed (1) to excited (5) and the dominance dimension ranging from 'dominated' (1) to 'in control' (5).

2.2.2.Resistive loads.

.In the present study, two linear flow resistors (Hans Rudolph, Inc.) were used: one of 5 cm/H_2O/l/sec, further called the low-intensity or CS-load, and another of 20 cm/H_2O/l/sec, further called the high-intensity or US-load. The CS load in the present study can be considered one just above detection threshold, as previous research has found an average threshold resistance of 1.63 cmH_2O/l/sec (SD = 1.06) and a sensitivity (A') of .82 (SD = .17) for a flow resistor of 4.01 cm/H_2O/l/sec in young, healthy participants (Bloch-Salisbury et al., 1998).

2.2.3. Apparatus and software.

Participants were seated in a comfortable seat. They wore a nose clip and breathed through a mouthpiece and bacterial filter connected to a Jaeger MasterScope with heated pneumotachograph (Hoechberg, Germany). A non-rebreathing valve mounted on the pneumotachograph separated inspired and expired air. A reinforced vinyl tube with

smooth interior (inner diameter: 3.5 cm; length 100 cm) connected the inspiratory side of non-rebreathing valve with a 4-way stopcock valve, enabling easy switching between the low-intensity load, the high-intensity load and unloaded breathing. A screen prevented the participant from seeing the 4-way stopcock and the loads. The respiratory drive module of the JaegerLab 4.67 program applied an inspiratory occlusion of 100 ms at the start of inspiration whenever the experimenter commanded this during the preceding breath. P100 occlusion pressure (kPa) was determined from the change in mouth pressure generated 100 ms following the start of inspiration. The flow signal (sampled at 250 Hz) changing from negative to positive values indicated the start of inspiration and triggered the closing of the shutter valve. Because the latter had a response time of 35–45 ms, linear curve fitting from the maximum value at the pressure curve (at 100 ms post occlusion) extrapolated the exact point in time where the inspiration had started. P100 was derived from the extrapolated curve at 100 ms following the (extrapolated) start of inspiration.

2.3. Procedure

Participants first read an information sheet explaining the purpose of the experiment. Next, they signed the informed consent form and completed the Fear of Suffocation scale. Following this, participants received instructions on how to hold the breathing apparatus and performed a practice breathing trial of 2 min to familiarize with the apparatus and the occlusions. Then, participants in the experimental group were presented both inspiratory resistive loads for three breaths and rated them on the affective

dimensions of valence, arousal and dominance, while in the control group this was only done for the low-intensity load.

During the subsequent conditioning procedure, participants watched a relaxing documentary (The Travelling Birds, Perrin, J., Cluzaud, J. and Debats, M., 2003); the experimenter explained that the movie intended to make the experiment less boring to the participants and that they were simply required to watch the movie with the breathing apparatus on.

Each trial comprised six breaths. Two types of trials were applied (A/B). They differed with respect to which of the six breaths was shortly occluded to measure the P100. In a first type (A trials), occlusions of 100 ms were applied at the onset of the third and the sixth breath. In the second type (B trials), similar occlusions were performed on the first and the fourth breath.

---------- Insert Figure 1 ----------

The experiment consisted of an acquisition phase (containing 12 A and 12 B trials) and a post-acquisition phase (8 A and 8 B trials). A and B trials were intermixed and presented in a fixed pseudo-randomized order, with the constraint that no more than two consecutive trials of the same type were allowed (see Figure 1).

During the acquisition phase, the experimental group received the low-intensity load (CS) during the first to third breath and the high-intensity load (US) during the fourth to sixth breath. The control group received the low-intensity load (CS) during the entire trial (6 breaths) (see Figure 1).

During the post-acquisition phase, the low-intensity load (CS) was presented for 6 breaths in both groups. Intertrial intervals (ITI) lasted for 50 seconds. During the first 30 seconds of these intervals, P100 was measured randomly in each one out of three breaths. After the experiment, participants rated the loads received during the conditioning procedure a second time on valence, arousal and dominance. Following this, they were fully debriefed.

3. Data Analysis and Design

In order to compensate for the high temporal variability of P100 measurements, P100 data of each participant were averaged across 4 consecutive trials of the same type (A/B), yielding a data matrix with 3 acquisition (Acq) blocks and 2 post-acquisition blocks for each participant.

As we did not expect and observe differences in P100 during the ITI compared to the 1st loaded breath (see Altose et al., 1976), these values were averaged and served as a 'baseline'-index of respiratory drive during unloaded breathing in subsequent analysis.

Analyses were performed using SPSS 20. Mixed regression models were used, as they provide a powerful and flexible approach to analyze repeated-measures data (Snijders and Bosker, 1999, West 2009). This mixed model approach allows to model the dependency between our repeated observations as a random effect by inclusion of a person-specific intercept and between-subject variance through random slopes. All contrasts were tested two-tailed and corrections for multiple testing are reported.

First, as an important premise of the current study was that the ventilatory response to inspiratory resistive loads is an increase in respiratory drive (Altose et al., 1976; Im Hof et al., 1986; Lopata et al. 1977; Poon, 1989), we checked whether the application of inspiratory resistive loads resulted in an increase in P100 in the present study. To this end, we tested whether (1) P100 during the low-intensity load (the average of the 3^{rd}, 4^{th} and 6^{th} loaded breath) was higher compared to unloaded breathing in the control group, and, whether (2) P100 during the high-intensity load (6^{th} loaded breath) was higher compared to unloaded breathing in the experimental group.

Second, a mixed model was run to explore general main and interaction effects in our data. This model included as fixed effects the categorical predictors Condition (Experimental / Control), Breath (ITI breath / 1^{st} breath / 3^{rd} breath / 4^{th} breath / 6^{th} breath), Block (Acq_1 / Acq_2 / Acq_3 / $Test_1$ / $Test_2$) and their interaction terms. Fear of Suffocation was included as a continuous predictor. The random part of the model consisted of a random (person-dependent) intercept and random slope for Breath and Block.

Third, our hypothesis that participants would learn to anticipatorily adapt to the strong load by increasing their inspiratory motor drive to the low-intensity load, was tested directly with planned contrasts. For each group separately, we tested whether (1) across acquisition blocks P100 during the 3^{rd}, 4^{th} or 6^{th} loaded breath increased relative to unloaded breaths, and (2) across post-acquisition blocks P100 during the 3^{rd}, 4^{th} or 6^{th} loaded breath increased relative to unloaded breaths. After inspection of the results, two

additional post hoc contrast were created. One tested a linear trend for the 6th breath across blocks for the experimental group. Another contrast investigated whether there was a group difference in the increase in P100 during the 3th loaded breath relative to unloaded breaths during the 1st acquisition block.

Finally, subjective ratings were analyzed in a repeated measure ANOVA with Load-type (low-intensity / high-intensity) x Time (pre- / post-experimental) as within subject variables. This was done for the experimental and the control group separately. Greenhousse-Geisser corrections were applied where appropriate. Significant effects will be further examined using Tukey tests for post-hoc comparisons. Only significant or relevant effects will be reported.

---------- Insert Figure 2 ----------

3.1. P100

3.1.1. Effect of respiratory load intensity

Across all blocks, the low-intensity resistive load (CS) was associated with an increased P100 compared to unloaded breaths ($t(40) = 2.338$, $p = .048^{a}$). Also the high-intensity resistive load (US) led to a strong increase in P100 compared to unloaded breaths ($t(48) = 5.555$, $p < .001^{a}$).

3.1.2. Mixed model

There was a main effect of Breath as both the low and high intensity load led to an increase in P100 compared to unloaded breathing ($F(4,88) = 8.207$, $p < .001$).

Furthermore, a marginally significat main effect of Fear of Suffocation (FoS) suggested that persons with higher scores on FoS scores tended to have a lower P100 (F(1,21) = 4.22, p = .053). The effect of Breath varied across Blocks, driven by the observed pattern in the experimental group across blocks (Fig. 2) (F(16,352) = 2.059, p = .010) and differed marginally between groups (F(4,88) = 2.422, p = .054). Also the three way interaction between Condition, Block and Breath was significant (F(16,352) = 1.939, p = .016), originating from an apparent initial increase in the 3rd and 6th breath, in the experimental group, that habituated across blocks (Fig. 2).

3.1.3. Planned contrasts

Experimental group

During the acquisition phase, participants generated a stronger P100 at the 6th loaded breath compared to unloaded breaths (t(77.44) = 7.021, p < .001[b]). Surprisingly, P100 of the 4th loaded breath did not differ from unloaded breaths t(77.44) = -.166, p = *1*[b]), whereas the P100 of the 3rd loaded breath, that is prior to the US, was significantly higher compared to unloaded breaths (t(77.44) = 2.833, p = .018[b]).

During post-acquisition, there were no differences in P100 during the 3rd, 4th or 6th loaded breath compared to unloaded breaths (t(116.707) = 1.019, p = .92; t(116.707) = 1.186, p = .714; t(116.707) = 1.531, p = .54).

Control group

During acquisition, neither P100 of the 6th loaded breath or the 4th loaded breath differed from the unloaded breaths (t(69.830) = 1.713, p = .273[b]; t(69.830) = -.292, p = 1[b]). After

correction for multiple testing, P100 during the 3rd loaded breath tended to be higher compared to unloaded breaths ($t(69.830) = 2.446$, $p = .051$[b]).

During post-acquisition, there were no differences in P100 during the 3rd, 4th or 6th loaded breath compared to unloaded breaths ($t(108.775) = 1.642$, $p = .306$; $t(108.775) = 2.004$, $p = .144$; $t(108.775) = 1.136$, $p = .774$).

3.1.4. Post hoc contrast

After visual inspection of the data, the significant three-way interaction (Condition x Block x Breath) was further explored (Fig. 2). During the first acquisition block, the experimental group displayed a significantly stronger increase in P100 during the 3rd loaded breath compared to the control group ($t(383.579) = -2.036$, $p = .042$[a]). Furthermore, the initial increase in P100 during the 6th breath of the experimental group decreased linearly across blocks ($t(182.293) = 4.541$, $p < .001$[a]).

3.2. Fear of suffocation

Groups did not differ on Fear of Suffocation scores ($t(22) = .288$, $p = .776$).

3.3. Subjective ratings

In the experimental group the US-load was rated higher on unpleasantness and arousal compared to the CS-load at both time points (see Table 1; $F(1,14) = 20.40$, $p < .001$ and $F(1,14) = 33.67$, $p < .001$). Ratings for unpleasantness and arousal of both loads were rated lower following compared to prior to the experiment ($F(1,14) = 11.45$, $p < .01$and $F(1,14) = 53.28$, $p < .001$).

In addition, the decrease in self-reported arousal was stronger for the US-load compared to the CS-load, as indicated by a significant load-type x time interaction ($F(1,14) = 6.14$, $p < .05$). A similar effect was found for the valence ratings, although only marginally significant ($F(1,14) = 3.50$, $p = .08$). The results for the control group are displayed in Table 1.

---------- Insert Table 1 ----------

4. Discussion

The present study applied two different inspiratory resistive loads in a conditioning paradigm to investigate interoceptive conditioning of the inspiratory motor drive (P100). In the learning (acquisition) phase, a small load applied for three breaths preceded a strong load for three subsequent breaths in the experimental group. The control group received six breaths in a row loaded with the small load. The post-acquisition phase consisted of six small loads in a row for both groups.

As expected, intensity-related effects of the resistive loads were found. An increased respiratory drive was generally observed with increasing load intensity: across breaths and blocks the low-intensity resistance load (CS-load) led to higher P100's compared to no load (ITI and 1st breath), and a strong P100 increase was observed during the high-intensity resistance load (US-load). These results confirm findings from previous studies that have investigated the effects of an increased airway resistance on the inspiratory muscle activity in normal subjects as assessed by inspiratory occlusion pressure. For example, Altose and associates (1976, 1979) found that in conscious humans P100 was

greater during mechanical loading than during normal free breathing and that mechanical loading resulted in augmented respiratory neural efferent activity unexplained by alterations in chemical stimulation. Also Kryger et al. (1975) and Gothe and Cherniack (1980) have reported that in normal awake subjects, added inspiratory resistance increased the inspiratory drive as measured by P100.

However, in contrast with our expectations, no such unconditional effects of inspiratory resistive loads were observed for the fourth breath. There was no increase in respiratory drive between the fourth breath and unloaded breaths. One speculative explanation suggested by the pattern in the control group is that our measure of respiratory drive is highly variable. Although they received the same load for six consecutive breaths for each block, there is a large variation in the mean P100. This variation might explain the observed overall effect (across blocks and breath) but the disappearance of this effect on a lower level (per breath per block).

Furthermore, evidence was found for a short-lasting anticipatory increase in inspiratory motor drive during the learning phase. There was an increase in P100 during the breath preceding the US-load during the first acquisition trials, which habituated across acquisition and extinguished during the post-acquisition phase.

The present data suggest that the short-lasting character of this anticipatory increase is related to participants' strong habituation to the US-loads across the experiment. This is evident from the left panel of Figure 2: Whereas participants from the experimental group show a strongly elevated P100 to the US-load (sixth breath, A trials) in the first acquisition block, this response strongly decreased during subsequent blocks. This demonstrates that participants initially reacted to the US-load by increasing their

inspiratory motor drive, but that this reaction pattern disappeared with repeated administrations. Also the self-reported data are in line with habituation: participants rated the US-load as less unpleasant and less arousing following as compared to prior to the conditioning procedure. The short-lasting anticipatory increase may therefore have disappeared due to the strong habituation to the US-load, as the US-load was no longer relevant to the participant.

In this respect, it is interesting to relate the present findings to studies that have investigated the ventilatory response to inspiratory resistive loads during different sleep stages and wakefulness (Morrell et al., 2000). A general finding from that literature is that the load compensation response is higher during wakeful states and REM sleep compared to non-REM sleep, suggesting that the load compensation response is primarily a behavioral response that is dependent on the background level of arousal. The loads applied in the present study were rather benign and applied for only a short time in a predictable way. Therefore, participants may have actually learned that the loads do not pose a homeostatic threat and can be disregarded. Thus, whereas participants initially increased their inspiratory effort to keep their ventilation at a constant level, they learned to let go this extra inspiratory effort later on in the experiment. Moreover, since the resistive loads are flow-dependent, participants may have experienced that by not augmenting their inspiratory motor drive, they experience less discomfort (less resistance to breathe against; see Kifle et al., 1997).

An important question in this context is the role of the fear system. Up till now, findings from our lab generally suggest that a fear response is necessary to establish feedforward learning in adult human breathing behavior. Anticipatory fear responses were observed

when using breathing loads and occlusions as US (Pappens et al., 2011; Pappens et al., 2012; Pappens et al., 2013). In addition, to these anticipatory fear responses a small change in breathing behavior was observed. In another study, we found that participants learned to decrease their ventilation in response to the CS+ odor before they were switched to the CO_2-breathing. In other words, breathing inhibition to the CS+ odor was established in acquisition (Fannes et al., 2008), which was interpreted as fear-related avoidance behavior.

The conclusion of the present study may be that short-lasting feedforward regulation of breathing develops but quickly disappears when the anticipated discomfort or the disturbance is benign, as it was the case in the present experiment.

Footnotes

(a) Corrected for multiple testing by a factor of 2.

(b) Corrected for multiple testing by a factor of 3.

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Table 1. Mean (SD) ratings on Valence, Arousal and Dominance prior and following the conditioning procedure for the experimental and the control group.

		Time			
		Prior		**Following**	
	Group	CS-load	US-load	CS-load	US-load
Valence	experimental	2.60_{ab} (0.83)	3.67_{c} (0.72)	2.20_{a} (0.94)	2.87_{b} (0.83)
	control	2.50_{a} (0.76)	-	2.79_{a} (0.89)	-
Arousal	experimental	2.33_{a} (0.82)	3.53_{c} (0.74)	1.67_{b} (0.49)	2.33_{a} (0.82)
	control	2.43_{a} (0.94)	-	2.50_{a} (0.85)	-
Dominance	experimental	3.53_{ab} (1.19)	3.07_{a} (1.03)	3.80_{b} (1.32)	3.33_{ab} (0.72)
	control	3.86_{a} (1.17)	-	3.50_{a} (1.09)	-

Note. Means with a similar subscript do not differ from each other on the $p < .05$ level (Tukey HSD test). Tukey HSD tests refer to within subject comparisons.

Figure 1.

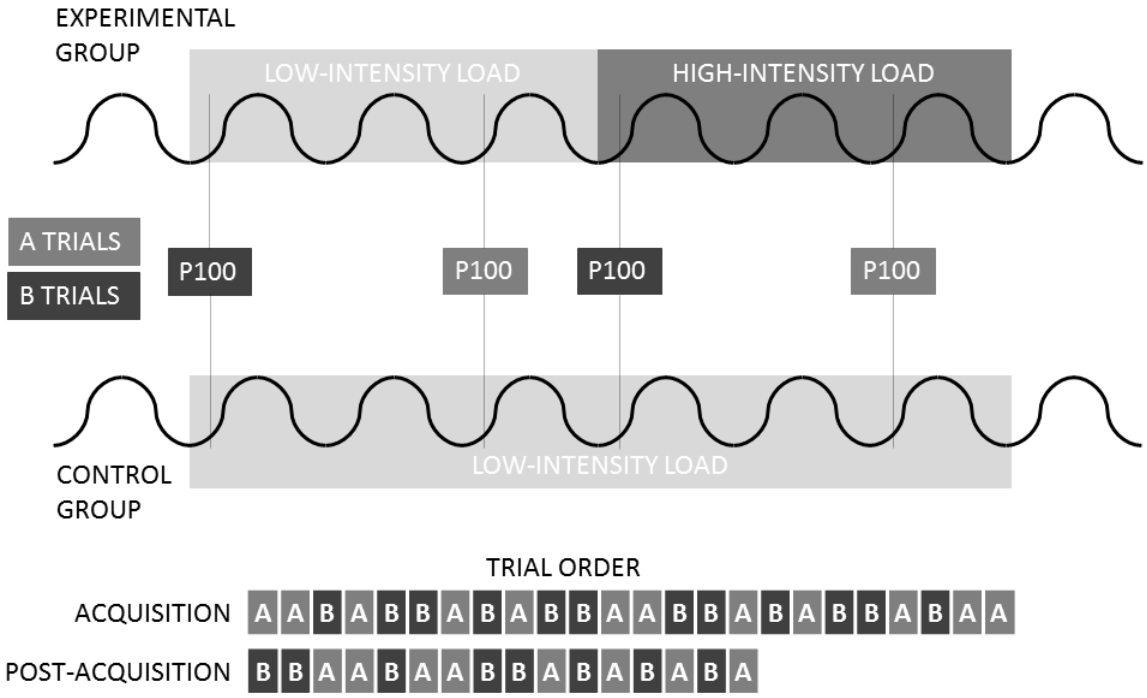

Figure 1. Overview of the paradigm. The black sinusoidal curve represents the respiratory cycles during a trial. The light grey zones indicate the application of the low-intensity load (CS-load). The dark grey zones indicate the application of the high-intensity load (US) during the acquisition phase. Note that during post-acquisition both groups received the low-intensity load for 6 consecutive breaths. The P100 boxes indicate the different time points at which P100 was measured (light grey backgrounds for A trials, dark grey backgrounds for B trials). The order of the different trials during acquisition and post-acquisition are displayed at the lower part of the figure.

Figure 2.

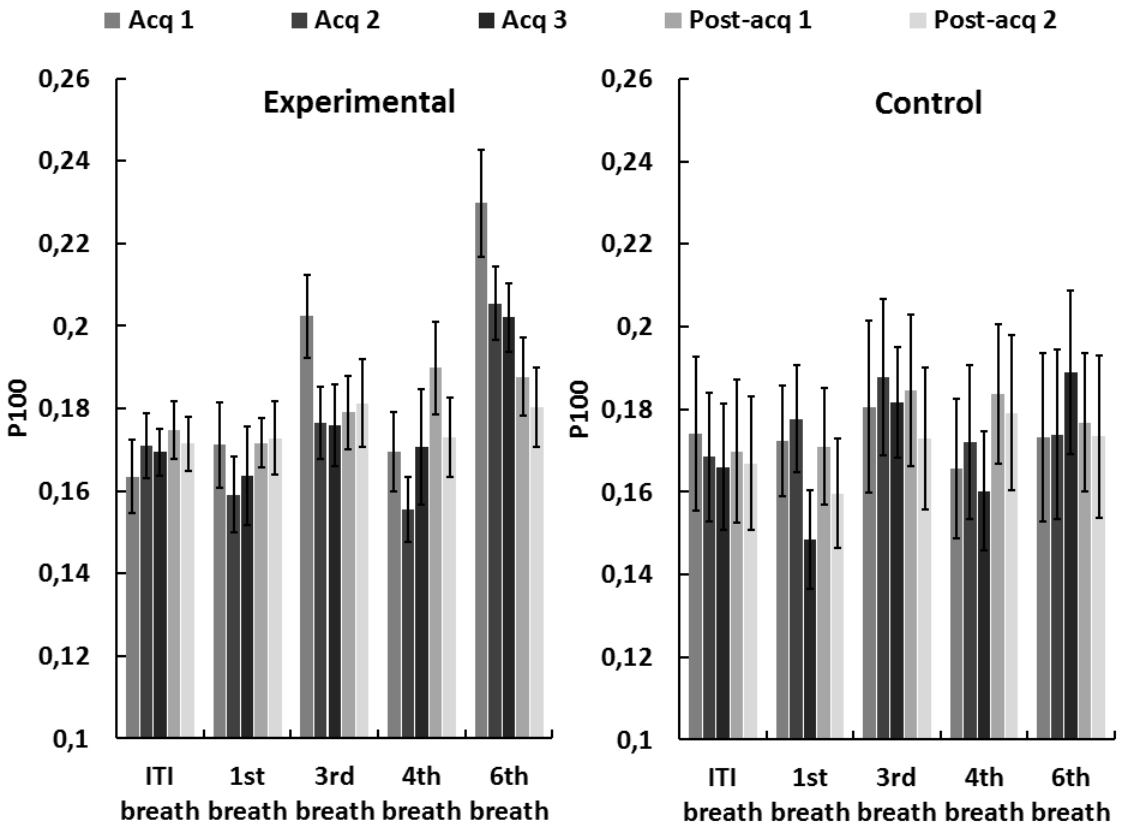

Figure 2. P100 for the different breaths across blocks for the experimental and control group respectively. Individual P100 averages were calculated on 4 consecutive trials of the same type (A/B), resulting in 3 acquisition blocks (Acq 1, 2 and 3) and 2 post-acquisition blocks (Post-acq 1 and 2). ITI = intertrial interval